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THE DEVELOPMENT OF IgM IN EVOLUTION; ITS ROLE IN PRIMITIVE ANIMAL SPECIES

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SUMMARY

The antibodies of immunoglobulin-type appear to be unique defence molecules of jawed vertebrates. Molecules of antibodies are formed by B-lymphocytes, in which the immunoglobulin genes undergo a series of chromosomal rearrangements and somatic mutations, thus generating antibodies with high affinity. This brief review brings in summary form, what is known about defence molecules in deuterostomian echinoderms and invertebrate chordates, and what a role the IgM plays in lower vertebrates.

INTRODUCTION

The defence of integrity of multicellular organisms is realised by means of very different humoral factors comprising a wide collection of molecules ranging from relatively simple lectins and acute phase type proteins which of example could serve C-reactive protein of horseshoe crab, from arthropod antimicrobial factors like attacins, cecropins and many others to oxidative enzymes to highly complex molecules of immunoglobulin superfamily such as specific antibodies and TCR. In *sensu lato*, also recognition molecules, cytokines, clotting factors and various signal molecules could be regarded as defence molecules.

GENERAL REMARKS

Immunoglobulin superfamily

The immunoglobulin multigene superfamily is in actuality an assemblage of smaller subfamilies, all supposingly derived from a common ancestral gene controlling a primordial immunoglobulin molecule, but diverged to separate functions by means of genetic processes.

Main unique features of molecules of immunoglobulin superfamily are multiplicity, close linkage, sequence homology and overlapping functions. Sequence relatedness to other member molecules and presence of ß-sheet globular domain (about 100-110 amino acids in size to either constant or variable domains of an immunoglobulin molecule) are main prerequisites for a molecule to be admitted to membership in the immunoglobulin superfamily (*Williams* and *Barclay* 1988).

Evolution of immunoglobulins

It is likely that molecules of immunoglobulin superfamily emerged from cell adhesion molecules (CAM) of plasma membranes known as cadherins

that first evolved to mediate interactions between cells (*Ohno*, 1996). They have been found in protostome invertebrates where they are more related to neural cell adhesion molecules (N-CAM) than immunoglobulins (*Hughes*, 1998).

The unequal crossing over, gene conversions and the residence on different chromosomes may be the primary force driving the evolution of the Ig superfamily. Possible mechanism is gene duplication from a primordial gene coding for about 100 amino acids forming a single Ig-domain. There are two forms of immunoglobulin molecule, the soluble antibody and the membrane-bound B-cell receptor for antigen which of immunoglobulin heavy chain C-terminal is encoded by a single gene.

It seems more probable that the emergence of the primordial immunoglobulin domain and its consequent duplication and diversification into L- and H-chains, and T-cell receptor domains had to occur within a relative short time span during evolution of gnathostomian ancestors, probably 10 million years (*Marchalonis* et al., 1998). It was proposed that the horizontal transfer of originally microbial genes RAG (recombination activating genes) and their incorporation into genomes of predecessors of jawed vertebrates could be an important evolutionary event which caused the duplication and rearrangement of the ancestral immunoglobulin gene elements, the capability of which could be utilised for the recognition of alien and the response to it (*Bernstein* et al., 1996b). This event can be considered, similarly to cosmologic hypothesis, as a "big-bang" for the onset of vertebrate adaptive immunity. The second key evolutionary event was invention to arrange gene segments V, D, J, and C.

From the evolutionary point of view, a candidate for primordial immunoglobulin molecule could serve $ß_2$-microglobulin consisted of 99 amino acid residues in single chain with one intrachain disulphide bond. It is ubiquitous on all mammalian cells with exception of red blood cells and a molecule with considerably high homology has been also found in many invertebrates. It was suggested that ancestral $ß_2$-microglobulin gene could diversified into a "primitive gene" in protostomes and into "primordial gene" in deuterostomes. From deuterostomian precursor, $ß_2$-microglobulin genes and immunoglobulin genes arose by rapid evolutionary diversification (*Shalev* et al., 1983). Similarly, the Thy-1 molecule is more primitive than immunoglobulin and MHC molecules. For the Thy-1 homologue was found in annelids, molluscs, and tunicates, it could be supposed that the Thy-1 gene may be evolutionary very closely related to the primordial gene for immunoglobulin and MHC in vertebrates (*Stewart*, 1992).

Generation of antibodies

The presence of multiple V genes in the genome is a fundamental *condicio sine qua non* for generation of antibody diversity. It is assumed that the evolution of immunoglobulin H-chain V region could last for 150-200 million years. Ontogenetic generation of the antibody repertoire is dependent on germline encoded V genes and D and J segments. Mammals generate antibody repertoire by many hundreds of V genes and several D and J segments (*Tonegawa*, 1983). In birds, the antibodies emerge by diversifying one or limited number of V genes and many pseudo-V genes through the process of gene conversion (*Reynaud* et al., 1985). The antibody repertoire of anuran amphibians seems to be rather re-

Table 1: The most important defence molecules of echinoderms

INVERTEBRATE TYPE FACTORS
(haem)agglutinins(C-type lectins), **(haemo)lysins, perforins,** (proteins)
involved in: agglutination, cell adhesion, cell lysis

FACTORS UNIQUE FOR ECHINODERMS
sea star factor, profilin (proteins)
involved in: inflammation, inhibition of macrophage, suppression of T-dependent mammalian immune response, signal transduction
antibody-like protein
involved in: inducible, complement-dependent lysis?

VERTEBRATE-LIKE FACTORS
IL-1-like, IL-2-like, IL-6-like, TNF-like, IFN-γR (proteins)
involved in: stimulation of proteosynthesis and phagocytosis, inflammation, cytotoxicity
C3-like (homologue to a vertebrate complement component)
involved in: opsonin ? primitive alternative pathway?

MEMBERS OF IG SUPERFAMILY
IL-1R, IL-6R (receptors)
involved in: ?

stricted but their V genes have been shown to have tendency to hypermutate (*Wilson* et al., 1992). The fish antibody repertoire is also limited and the affinity maturation during the humoral immune response is not fully manifested (*Witzel* and *Charlemagne*, 1985).

THE VECTORS OF HUMORAL IMMUNITY OF DEUTEROSTOMIAN INVERTEBRATES

The echinoderms

The members of phylum *Echinodermata* are thought to have originated from a common ancestor with chordates. Although their larvae are bilaterally symmetrical, they gain the secondary pentameric radial arrangement of their bodies.

Immunocompetent structures

The echinoderms are not endowed by any structures or differentiated immunocompetent organs. Phagocytic sessile and dispersed coelomocytes could be analogised as the vertebrate "reticuloendothelial system." The axial organ has similar poietic and phagocytic functions to the spleen and lymph node, respectively. Its cells are differentiated into phagocytic and lymphoid-like cells being divided into B-like and T-like cells.

Defence molecules

Several interesting humoral factors playing a role in echinoderm humoral defence have been described, namely (haem)agglutinins (lectins), complement-like and lysozyme-like molecules (Table 1). Some molecules act as perforins or in signal transmission like profilins (for review see *Matranaga*, 1996).

Sea star factor of *Asteria forbesi* (*Prendergast* and *Suzuki*, 1970) has significant suppressive effects on the development of T-dependent antibody secreting clones by preventing lymphokine secretion (*Kerlin* et al., 1994).

In *A. forbesi*, the presence of regulatory cytokine-like molecules, an IL-1-like and IL-6-like proteins with conserved amino acid sequence when compared to mammalian interleukine counterparts, was described (*Beck* and *Habicht*, 1996). In addition, cytokines released by axial organ T-like cells with properties similar to vertebrate IL-1 and IL-2 (*Leclerc*, 1996) and cell surface structures resembling human receptors for TNF, IFN-γ, IL-1, and IL-6, were found in *A. rubens*.

The axial organ B-like cells of sea star, in many aspects similar to vertebrate lymphocytes, were found to react to the antigenic challenge by the secretion of a specific antibody-like protein capable of lysis of haptenated erythrocytes in the presence of complement (*Delmotte* et al., 1986). Compared to the immunoglobulin of lower vertebrates, this antibody-like substance is structurally simpler, not having L- and H-chains.

On the basis of molecular analyses, a primitive homologue of complement system composed of at least one component (C3) and a receptor was identified in sea urchin *Strongylocentrotus purpuratus*. This system might serve as a platform onto which the Ig gene system was latter added and resulted in the sudden expansion of both systems in higher vertebrates (*Smith* et al., 1996).

In conclusion, echinoderms like other invertebrates secrete various non-specific humoral immune substances into their coelomic fluid. Despite of this fact, the echinoderms have developed an efficient immune strategy with some interesting similarities in fundamental defence features to the vertebrates. The presence of receptors for IL-1 and IL-6, the members of Ig superfamily, and cytokine-like factors such as IL-1, IL-2, IL-6 or TNF provides support for a continuity of rearranging Ig molecules and immunoregulative molecules in at least deuterostome phylogeny (*Legac* et al., 1996).

THE VECTORS OF HUMORAL IMMUNITY OF INVERTEBRATE CHORDATES

The tunicates

The tunicates are considered to be the most primitive present-living members of phylum *Chordata*. Owing to their evolutionary position they have attained attention of comparative immunologists as organisms in which first traces of adaptive immunity could be found.

Immunocompetent structures

First distinct mesodermal-derived haemopoietic structures have developed in ascidians. Accumulations of stem haemoblasts are located in mesenchymal tissues, diffused or structured into “lymph nodules“ along the digestive tract. Structural composition of pharyngeal wall, where mutual interactions among ectodermal epithelium, mesenchymal tissue, and endoderm took place, could serve as a background for later developmental potential for the thymic emergence in vertebrates. The mesenchymal origin of blood cells, of which one type is almost indistiguishable from vertebrate lymphoid cells and the organisation of nodules in the tight vicinity with the pharyngeal region and the gut, are common features shared with all vertebrates.

Defence molecules

Similar to protostomian invertebrates, substances involved in immune system of tunicates are prevailably haemagglutinins (lectins, some of them

Table 2: The most important defence molecules of tunicates

INVERTEBRATE TYPE FACTORS

(haem)agglutinins C-type lectins), opsonins (various)

involved in: agglutination, cell adhesion and migration, LPS-binding, opsonisation, stimulation of cell proliferation, recognition, phagocytosis

FACTORS UNIQUE FOR TUNICATES

lamellarins (poly-aromatic alkaloids), **crucigasterins** (poly-unsaturated amino-alcohols), **dicyclamids, didemnins, ulithiacyclamids, patellamids** (cyclic peptides), **halocyamins** (dihydrotryptamins), **substance Ete** (cytokine-like), **ecteinascidins,** eudistomins (β-carboline derivatives)

involved in: antiviral, antimicrobial and antineoplastic activities, cytotoxicity, stimulation of phagocytosis

complement control superfamily factors

involved in: ?

VERTEBRATE-LIKE FACTORS

IL-1α, IL-1β, (C-type lectins)

involved in: stimulation of cell proliferation. co-mitogenic

MEMBERS OF IG SUPERFAMILY

Thy-1 homologue, Lyt-2/3 (CD8) homologue

involved in: ?

even more similar to plant lectins rather than animal lectins), various antimicrobial and antiviral substances, opsonins, enzymes, poly-aromatic alkaloids or cyclic peptides, and cytokine-like substances (Table 2). Cytokine-like protein with the similar activity to IL-1 was found in a row of tunicate species (*Beck* et al., 1989; *Raftos* et al., 1992). The opsonin isolated from *Styella clava* is functionally and physicochemically similar to acute phase proteins of mammals (*Kelly* et al., 1992). Analyses of urochordate cDNA revealed a 50% identity with the short consensus repeats of the human complement factors, most of all factor H, apolipoprotein H, or complement receptors type 1 and 2 (*Pancer* et al., 1995). Hypothetically, this polymorph sequence might represent and ancestral molecular prototype in evolution of complement control protein superfamily.

In 1968 F.M. Burnet suggested that haemagglutinins documented in tunicates might be forerunners of vertebrate immunoglobulins. It has been shown by *Rosenhein* et al., (1985) that some components of the plasma of *Halocynthia pyriformis* and *Boltenia ovifera* react with antisera against shark immunoglobulin 7S heavy chain. Therefore, the possibility cannot be ruled out that these plasmatic proteins could represent the ancestral precursor of immunoglobulin molecule. In 1984 a molecule cross-reactive with the shark μ-chain (μCRM) was isolated from the haemolymph of *Pyura hausteria* and *B.ovifera*. On the basis of immunochemical data and sequence homologies, the relationship of tunicate μCRM to Ig is indicated (*Schluter* et al., 1994). The occurrence of a Thy-1 homologue, a simple member of immunoglobulin superfamily, was ascertained in a solitary tunicate *S. clava* (*Mansour* and *Cooper*, 1984). Testing of tunicate haemocytes for the possible expression of Lyt antigens revealed a homologue of the murine Lyt2/3 complex (CD8), another molecular member of immunoglobulin superfamily (*Negm* et al., 1992).

Table. 3: The most important defence molecules of cyclostomes

INVERTEBRATE TYPE FACTORS
haemagglutinins (C-type lectins), **opsonins, bactericidins** (various), **haemolysins** (proteins)
involved in: agglutination, cell adhesion, antimicrobial, cell lysis

FACTORS UNIQUE FOR CYCLOSTOMES
?

VERTEBRATE-LIKE FACTORS
C3, C4, C5 (complement components)
involved in: alternative pathway, devoid of lytic action

MEMBERS OF IG SUPERFAMILY
?

Conclusively, the tunicates have developed immune phenomena like inflammation, genetically controlled non-fusion (colony-specific) reaction or allogeneic recognition and rejection of tissue grafts, which strongly resemble those of vertebrates. Although tunicates do not have some any antibodies or complement, the presence of cytokine-like substances suggests a possible common ancestry of some constitutive defence substances in advanced vertebrates with defence factors of invertebrate chordates or even invertebrates. The possibility cannot be ruled out that some of these molecules are structurally very close to primordial immunoglobulin molecule. The presence of Thy-1 and Lyt2/3 homologues, the members of Ig superfamily, documents tunicate key position in evolutionary pathways to vertebrate immunoglobulins.

THE VECTORS OF HUMORAL IMMUNITY OF JAWLESS VERTEBRATES

The cyclostomes

The members of extant two orders of cyclostomes, hagfish and lampreys, represent the most primitive species of the vertebrates. Comparative immunologists do not often consider the fact that the myxinids profoundly differ from petromyzontids in many embryological, anatomical, physiological and biochemical features. The lampreys could be categorised as a sister group of jawed vertebrates, whereas the hagfish have evolved independently.

Lymphoid tissues and organs

Equivalents of bone marrow, spleen, and lymph nodes may be preferentially attributed to pronephros and supraneural body, typhlosole, and cavernous body, but the distinct diversification onto primary (thymus, Bursa of Fabricius) and secondary immune organs and tissues seems to be absent (*Zapata* and *Cooper*, 1990).

Defence molecules

Main vectors of humoral immunity in cyclostomes are naturally occurring haemagglutinins and haemolysins and proteins homologous to the C3, C4, and C5 vertebrate complement components (Table 3). Cyclostome complement resembles an alternative pathway. It is devoid of significant lytic action, but

acts as the essential phagocytic factor (*Nonaka* and *Takahashi*, 1992; *Fujii* et al., 1995). Although some authors (e.g. *Varner* et al., 1991) had reported sequence similarities between a hagfish protein believed to be an immunoglobulin, molecular sequence analyses clearly proved it was a serum complement protein (*Ishiguro* et al., 1992). Moreover, the presence of genes for TCR, MHC and RAG in either hagfish or lampreys has not been described (*Klein* and *Sato*, 1998).

Mucosal immunity

Ancient agnathans were probably microphagous animals. Present-living cyclostomes are secondary specialised to parasitic life. Primitive foci of lymphoid cells (e.g. in branchial region specialised in trapping of particles) and lymphohaemopoietic aggregations accompanying veins of the gut (e.g. in the intestine submucosa of hagfish and typhlosole of lampreys) function analogically as GALT of endotherms (*Tanaka* et al., 1981). Nevertheless, neutrophil leukocytes form the most abundant cell population of the hagfish intestine. In lampreys, the plasmacytes with conspicuous morphology were distinguished in the typhlosole. It seems more probable that the lymphomyeloid intestinal tissue has poietic rather than immune functions thus resembling bone marrow.

Conclusively, both hagfish and lampreys lack major immune tissues and organs like thymus, spleen, and true hierarchised GALT. It is not definitively known, if the T and B cell dichotomy exists. Moreover, the absence of immunoglobulins implies that the present-living species of cyclostomes are endowed by the invertebrate type of constitutive immunity. From comparative immunology standpoint, and contrary to previous opinions, cyclostomes are clearly demarcated from other vertebrates.

THE IMMUNOGLOBULINS OF JAWED VERTEBRATES

The cartilaginous fish

The members of cartilaginous fish are divided into two main assemblages, the elasmobranchs comprising modern sharks, skates and rays, and the holocephalans, the chimeras. Studies of immunity have focused on mainly carcharhine sharks which arose approximately 60 million years ago, and in present forming more than 50% of all extant elasmobranchs, some representatives of related squalomorphs, more distant and older groups of heterodontid sharks, rays and chimeras.

Lymphoid tissues and organs

The thymus is multilobulated organ clearly consisting of cortex and medulla. The spleen has been proved to be major organ of the antibody formation. Lymphomyeloid structures, which may substitute the bone marrow and lymph nodes, such as organ of Leydig or epigonal organs are located in the oesophagus and in the gonads, respectively. Lymphocytes, plasmacytes, and macrophages, similar to those of all jawed vertebrates, have been described in these animals (*Zapata* et al., 1996).

Immune molecules of immunoglobuliny superfamily

All representatives of cartilaginous fish studied up to the present possess genes for expression of immunoglobulins, T-cell receptors (α/β and γ/δ), MHC class I and II, and RAG-1 (*Marchalonis* et al., 1998) (Table 4).

Table 4: The most important defence molecules of cartilaginous fish

NATURAL FACTORS

(haem)agglutinins (C-type lectins), **(haemo)lysins** (lytic enzymes), **acute phase proteins, complement** (both pathways), **squalamine** (aminosterol antibiotic)

involved in: agglutination and lysis, opsonisation, regeneration and wound healing, phagocytosis and chemotaxis, inflammation, antibacterial and antifungal activity

CYTOKINES

?

IMMUNOGLOBULIN SUPERFAMILY

IgM, (pentamer, dimer and monomer molecules), **IgNARC** (chimeric molecule), **NAR, IgW** (orthologue of IgNARC), **IgR (IgX), TCRα/β TCRγ/δ, MHC class I, class II, β2-microglobulin**

involved in: humoral immunity (mucous and serum antibodies), recognition, transplantation immunity

Immunoglobulins

Cartilaginous fish respond to antigen challenge by producing of elevated levels of specific antibodies. Secondary and repeated immunisations do not increase either quantity or the affinity of antibodies. It may be consistent with the restriction of antibodies to the IgM isotype and also with the unique arrangement of immunoglobulin gene segments. IgM molecule is a pentamer (17S -19S) of the basic structure H_2L_2 and a H-chain composed of four C domains. In the serum of some shark species, monomers and dimers of IgM have been found. For a survey of older studies of shark immunoglobulins see *Vêtviçka* and *Síma* (1998). In 1996, two new non-IgM isotypes were reported in nurse shark, IgNAR and IgNARC (Ig new antigen receptor from cartilaginous fish). The IgNARC appears to correspond to IgW of sandbar shark (*Bernstein* et al., 1996a; *Greenberg* et al., 1996). The IgNAR molecule is a dimer consisting of a modified ω H-chains comprising one V and five C domains but no L-chains. The IgW molecule overall resembling μ chains of IgR (IgX), another isotype described in skates and rays and mammalian IgM, is considered to be a surface molecule of shark B cell. Unlike any other H-chain-type molecule, it is comprised of six C domains. It is suggested that IgW may retain ancestral features of immunoglobulin molecule.

In contrary to mammals, chondrichthyans possess two distinct sets of H-chain V regions: Vω, which occur only in sharks and may be in other species of cartilaginous fish, and the V region associated with μ chains which could be a derivative of common ancestor shared with V_H domains of all jawed vertebrates. The shark Vμ shares greater identity to mammalian counterpart than it does Vω taken from the same species. The evolutionary separation of constant domains Cω and Cμ had also to be a very ancient event in the history of cartilaginous fish because they share very low identities when compared among different species. A different situation exists in skates where an 18S IgM molecule and 8.9S IgR molecule have been described. The IgR is produced by the plasma cells in a different way from those secreting IgM (*Tomonaga* et al., 1984).

V, D, J, and C gene segments occur in individual clusters apparently unlinked to one another, rather than in the translocon arrangements typical for

mammals (*Hinds* and *Litman*, 1986). The sharks, rays, and skates are unique in having distinctive multicluster or multiple locus arrangement for the H-chain of their IgM and IgR, in which units of V_H-D_H-D_H-J_H-C_H are many times (approximately 100 times) repeated on the chromosome. L-chain genes show the similar type of repeated multicluster gene organisation with V_L-J_L -C_L. Sharks possess three classes of L-chains: κ, λ and a type restricted to them. About at least three distinct families of λ chains are organised into 200 separate clusters of V_L segments, each of which contains an individual V, J and C segment (*Shan* et al., 1996). This configuration of the IgH and the IgL loci is possible reason for the restriction of combinational diversity, even though the number of clusters may be very large. Restriction of antibody diversity is caused by joining of V_H-D-J_H segments in the germ-line and by unique type of rearrangement which takes place within a cluster but not between clusters. The loci for IgNAR and IgNARC are small with a few genes arranged in clusters. Up to date there is no information on the IgW locus (for rev. see *Warr*, 1995).

Mucosal immunity - the jaw hypothesis

"The new ability to bite and swallow food by animals with the jaw would have caused increased frequency of physical injuries in the wall of digestive tract (oesophagus, stomach and intestine) of those primitive jawed fishes, which eventually led to the development of adaptive immunity." (*Matsunaga* and *Andersson*, 1994; *Andersson* and *Matsunaga*, 1996). It could be easily imagined that these animals explored all available mechanisms which they had inherited from their ancestors for watching and defending their digestive tract. Thus, it is understandable that from the first jawed animals, the GALT has gained its main immune importance. In contrast to cyclostomes, true small lymphoid nodule-like or massive accumulations of granular, macrophage, lymphoid, and plasma cells including antibody forming cells can be seen in a spiral valve or duodenal lamina propria and gut epithelia. IgM has been detected in the gut mucus and bile in quantities similar to serum levels (*Hart* et al., 1987).

Generally, the cartilaginous fish are the first vertebrates exhibiting true adaptive immunity. For the first time in the phylogeny of vertebrates, the major immunocompetent tissues are structuralised as distinct organs. On the other hand, neither true effector functions of T lymphocytes nor T and B co-operation have been described. The study of cytokines have, thus far, been omitted in chondrichthyans. Surprisingly, the IgM is not the primordial immunoglobulin isotype and the arsenal of immunoglobulin classes is greater than previously supposed. Nevertheless, there is a lack of affinity maturation of antibodies during the immune response.

The bony fish

Because of being under the continuous selective pressure of aqueous environment, the fish retained a high degree of conservatism in their body plan which is shared by all fish taxa independently of their kinship. That is the reason why the seemingly compact taxa of fish are more heterogeneous and phylogenetical distances among them are much more greater than among the mammals. A very limited sample of fish has been studied from the point of their immunity. Substantial knowledge is available only on fishes of commercial importance, whereas the deep-sea or pelagic fishes are omitted. Despite these limitations we are more or less able to deduce the general pattern of their immune capacities.

Table 5: The most important defence molecules of bony fish

NATURAL FACTORS

(haem)agglutinins (C-type lectins), **(haemo)lysins** (lytic enzymes), **opsonins** (various), **granulins** (epithelin family growth factors), **lipoxins** (eicosanoids), **C-reactive protein, amyloid P** (pentraxins, acute phase proteins), **complement** (both pathways)

involved in: agglutination and lysis, opsonisation, regeneration and wound healing, phagocytosis and chemotaxis, inflammation

CYTOKINES

CSF, IL-1α/β, IL-2-like, IFN-like (IFN-α?, IFN-β), TGF-β, TNF-α

involved in: immune reactions (antiviral), immune processes regulation, growth factors, cell proliferation stimulators

IMMUNOGLOBULIN SUPERFAMILY

IgM (tetramer and monomer molecules), **IgN**, **IgD-like?** (chimeric molecule), **TCRα/β, TCRγ/δ, MHC class I**, **class II, β2-microglobulin**

involved in: humoral immunity (mucous and serum antibodies), recognition, transplantation immunity

Lymphoid tissues and organs

Like in chondrichthyans, the thymus and spleen are fish major lymphoid organs. The thymus cortex and medulla are lacking in most species. There is only limited knowledge on the maturation of thymocytes and the origin of T-cell specificity in immune response. For the first time in the phylogeny, the structures similar to Hassall's corpuscles have been reported in some species. The fish spleen is a main site where formation of antibodies takes place. The absence of germinal centres is compensated by melano-macrophage centres and ellipsoid sheets. The kidney and particularly the well-developed GALT serve as analogues of bone marrow and lymph nodes. They represent major sites of haemopoiesis together with processing of antigens, and production of antibodies. The B and T cell dichotomy in fish has been documented by means of monoclonal antibodies (*Koumans-van Diepen* et al., 1995; *Rombout* et al., 1997).

Immune molecules of immunoglobulin superfamily

As in cartilaginous fish, in all species of bony fish studied, the genes for immunoglobulin, TCRα/β TCRγ/δ, β2-microglobulin, MHC I and MHC II, and RAG-1 were documented (*Matsunaga* and *Rahman*, 1998). It was suggested that fish TCR may be close in shape to the ancestral molecule (for review see *Press*, 1998) (Table 5).

Immunoglobulins

Bony fish, unlike cartilaginous fish, synthesise only IgM class antibody, which is tetrameric (in contrast to the pentameric structure of other gnathostomes) without J-chain. Numerous studies have confirmed the fish H-chain is rearranged from V_H, D_H, J_H and C_H genes, and organisation of these genes is variable (*Warr*, 1995). The genomic organisation of IgH-chain locus in fish is of "translocon type" found in amphibians and mammals, as V_H, D_H, and J_H exons are located in separate regions undergoing somatic reorganisation and splicing with a single C_H element (*Wilson* et al., 1995). Like in mammals possessing more than 100 V_H genes grouped in 7 families in the human and 14 in the mouse, from at least 2 to 11 V_H families, in which the number of V_H

genes is well over 100, were identified in teleost fish studied in this respect. Fish L-chains differ from H-chains in having only single C region domain in addition to the V domain. Gene segments for L-chains show the multicluster type of organisation, with V_L, J_L and C_L segment represented in each cluster, D region is absent. Fish L-chain population is heterologous, the L-chains fall into a number of distinct structural types. Whether of not fish L-chain types could be analogised to mammalian κ and λ chains is to be verified. A novel chimeric IgH chain, partly homologous to IgG was reported recently (*Wilson* et al., 1997). It contains a rearranged V domain, the first C domain of μ, and seven C domains encoded by δ gene homologue. On the basis of these analyses, it could be hypothesised that the IgD was primarily an ancient immunoglobulin molecule present in predecessors of both mammals and bony fishes. To be able to make any conclusion about this surprising finding, one should wait for an independent confirmation. Some species of fish possess low-molecular weight immunoglobulins, a monomeric form of IgM (*Wilson* and *Warr*, 1992), a smaller form of monomeric IgM designated IgM(ΔFc) (*Clem*, 1971), and IgN in the lungfish (*Marchalonis*, 1969).

Mucosal immunity of fish

Fish are the first vertebrates in which specific secretory immunity was observed. The skin mucus forms an important barrier in prevention of penetration of pathogenic bacteria and fungi. Besides immunoglobulins, the non-specific factors like (haemo)lysins, (haem)agglutinins (lectins), lytic enzymes, lysozyme, C-reactive protein, and complement are also present.

In some species, intraperitoneal injection of various antigens results in the formation of specific antibodies not only in the blood serum, but also in skin mucus and in the lamina propria of the gut (for review see *Kaatari* and *Piganelli*, 1996). In most studied species of fish, whole complex of GALT functionally approximates the intestine barrier of mammals. The intestinal wall is infiltrated by lymphocytes, granulocytes and other types of cells often aggregated in the mucosa and lamina propria, even if the structures resembling typical Peyer's patches are lacking. Similarly, lymphocytes secreting immunoglobulins occur in skin and gills. In chondrosteans (paddle fish, sturgeon) and in sarcopterygian lungfish, the spiral valve still develops and it is similarly infiltrated by lymphoid cells, often in aggregates, as in cartilaginous fish. In more advanced teleosts, scattered lymphocytes, plasmacytes granulocytes and macrophages, and their aggregates occur in and under the intestinal epithelium particularly in the posterior part of the gut (hind gut). The epithelial cells of the gut may play the same role in food antigen collecting as M cells of Peyer's patches. In the seahorse, the secondary specialised microphagous fish, which has altered the mouth structure and feeding habits, no lymphoid cells or lymphoid cell aggregates in the lamina propria were found, while they are retained in kidney or other locations of their body (*Matsunaga* and *Rahman*, 1998).

In summary, the present-living species of bony fish have developed proper modification of adaptive immunity during million years of their divergence from hypothetical common gnathostome ancestors. The organisation of their lymphoid tissue represents a further advance. The bony fish have retained some fundamental immune mechanisms from the epoch of their ancestral origin, from which as an example could serve preponderant tetrameric IgM molecule. On the other hand, the presence of regulatory cytokines, the

IFN-γ, Il-1 and IL-2-like factors, and macrophage activating factor (for review see *Vêtviçka* and *Síma*, 1998) could suggest that fish cellular and humoral immune processes tightly approximate to those of more advanced tetrapods.

CONCLUSION

The vertebrate immune system did not spring out of nothing. The ancestral molecules which gained later in phylogeny the function of specific immune humoral factors had to be already present in invertebrate animals forming a progenitory assemblage from which the predecessors of deuterostomian chordates have evolved. It might be namely the primitive immunoglobulin molecule, which in echinoderms, invertebrate chordates, and vertebrate cyclostomates did not developed due to a lack of a structuralised lymphoid tissue providing the suitable internal micro-environment for the induction of molecular variability. This morpho-functional background for these events started to develop together with the emergence of jaws and the fundamental body plan of gnathostomean vertebrates. Obviously, the adaptive immune system is an invention of jawed vertebrates. On this stage of evolution, first true immunoglobulin molecules appears: NAR, IgNARC, IgW, IgR, and IgM. Only the IgM seems to become the universal class of immunoglobulins for all advanced vertebrates.

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LITERATURE

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